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Serving Ontario through veterinary science, technology transfer, outbreak investigation and animal health surveillance

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Coxiella burnetii Abortion in Dairy Goat Herds

Jocelyn Jansen, Veterinary Science and Policy Unit and Paisley Canning, Summer Student, OMAFRA

Q fever, caused by the rickettsia *Coxiella burnetii*, is a zoonotic disease of concern throughout the world. Most human infections with *C. burnetii* are asymptomatic or result in mild flu-like illness. Occasionally, more serious signs can include pneumonia, hepatitis and endocarditis. Wild and domestic mammals, birds and arthropods are all reservoirs of the infection. Cattle, sheep and goats are considered the main source of infection for humans. *C. burnetti* is shed in large numbers in placentae, uterine fluids, milk and feces of infected animals and is extremely stable outside of the host. Infection in animals (and humans) may occur through direct contact with infectious materials or by inhaling contaminated particles. Serological studies suggest that infection may be widespread, particularly in ruminants and the agent is considered endemic in Ontario. Although infection in ruminants is common, clinical disease is rare. Abortions and stillbirths due to *C. burnetii* occur late in gestation in goats and sheep. This is a result of severe damage to the placenta, necrosis of the cotyledons and thickening of the intercotyledonary areas. In 2008, 15 cases of *C. burnetii* abortion (13 caprine, 2 ovine) were identified at the Animal Health Laboratory. Between 2003 and 2007, four to eight cases per year were identified.

In a recent French study by Rousset *et al.*, eight dairy-goat herds experiencing *C. burnetii* abortions were examined and the proportions of animals shedding *C. burnetii* among those that had aborted and those that had not were described. Aborting and non-aborting goats were monitored by polymerase chain reaction (PCR) for *C. burnetii* shedding 15 and 30 days after the abortion episodes or during the last month of gestation for non-aborting goats. PCR analyses of all samples showed that, on any day, 70% (35/50) of the aborting and 53% (37/70) of the non-aborting goats were positive. *C. burnetii* was shed into vaginal mucus, feces, and milk of 44%, 21%, and 38%, respectively, of goats that aborted and 27%, 20%, and 31%, respectively, of goats that delivered normally. The number of shedders was not significantly different between the two groups. Asymptomatic (non-aborting) goats still shed *C. burnetii*, making them an important source of environmental contamination.

A number of serological tests (ELISA, complement fixation, indirect immunofluorescence assay) have been developed to identify antibodies to *C. burnetii*. A positive serological test in an animal, though indicative of infection at some time, does not necessarily signify a current abortion/stillbirth problem. Rousset *et al.*, did not find agreement between PCR (shedding) and antibody (serological) response results. Among the PCR-positive goats, 24% to 39% were seronegative depending on the test used.

Prevention strategies and biosecurity protocols have an important role in reducing the spread of *C. burnetii*, within a farm, between farms and to

(Continued on page 3)

humans. This is particularly important now with expansion occurring in the Ontario goat industry. Indirect and direct exposure of non-infected animals to infected animals must be avoided. Shedding can last up to six weeks and, in one study (Berri *et al.*), some goats continued to shed the organism for up to four months or for two successive parturitions after the initial outbreak. In Europe, the vaccine COXEVAC (Ceva Santé Animale, Libourne, France) is used extensively, and is reported to effectively reduce abortions and shedding of *C. burnetii*. The vaccine is not available in Canada but veterinarians can fill out an Application for Permit to Import Veterinary Biologics into Canada, through the Canadian Food Inspection Agency (CFIA). At this time the vaccine can not be imported for prevention purposes. Permission is only granted for herds with active outbreaks.

Producers and veterinarians are encouraged to submit placentas and feti from aborted females to a diagnostic laboratory to diagnose the causative agent and start appropriate treatment and control strategies. Considering all abortions in small ruminants to be potential zoonoses until otherwise identified, is a wise precaution.

Rousset E, Berri M, Durand B, Dufour P, Prigent M, Delcroix T, Touratier A, Rodolakis A. Coxiella burnetii shedding routes and antibody response after outbreaks of Q fever-induced abortion in dairy goat herds. Appl Environ Microbiol 2009; 75(2):428-433.

McEwen B, Slavic D, Ojkic D, DeLay J, Cai H, Stalker M. Selected zoonotic pathogens and diseases identified at the AHL, 2008. AHL Newsletter 2009; 13(1):3.

McEwen B, Slavic D, Ojkic D, DeLay J, Cai H. Selected zoonotic pathogens and diseases identified at the AHL, 2003-2007. AHL Newsletter 2008; 12(1):3.

Smith MC, Sherman DM. Goat Medicine. Philadelphia: Lea & Febiger, 1994:422-423.

Berri M, Rousset E, Hechard C, Champion JL, Dufour P, Rodolakis A. Goats may experience reproductive failures and shed Coxiella burnetii at two successive parturitions after a Q fever infection. Res Vet Sci 2007; 83:47-52.

Investigation of the Role of *Chlamydophila* spp. and *Coxiella burnetii* in Caprine and Ovine Abortions

Hugh Cai, Josepha DeLay, Murray Hazlett, Beverly McEwen, and Durda Slavic, Animal Health Laboratory (AHL), University of Guelph

The AHL has, through the Animal Health Strategic Investment Project, started investigations into the role of *Cp. abortus* and *C. burnetii* in small ruminant abortions in Ontario. Investigations will determine if abortion is related to pathogen loads using quantitative real-time polymerase chain reaction (PCR). Immunohistochemistry (IHC) for both of these agents will also be performed for validation purposes (**Figure 1**).

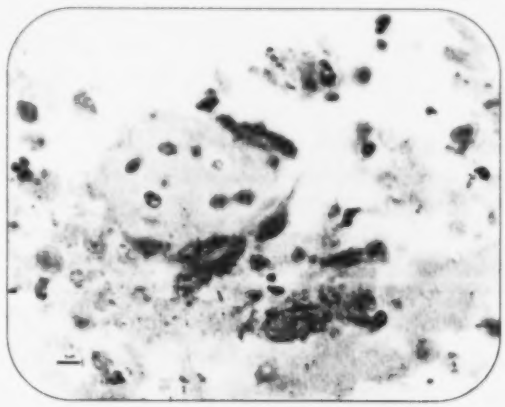

Figure 1. *Chlamydophila abortus* IHC. Caprine placenta. Organisms stain bright red. Bar = 5 microns.

Practitioners should note that, until May 2010 or further notice, necropsies on aborted lambs and goats will be paid for with study funding, and there will be no charge to the owner or veterinarian ***provided the placenta is submitted*** either with the fetus (preferably) or alone. Fetuses submitted without a placenta will incur normal charges as listed in the AHL fee guide.

Be on the Look-out for an Unusual Mucosal Disease in Slobbering Sheep.

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Between April 2007 and April 2009, an unusual and dramatic erosive mucosal disease was diagnosed in lambs submitted for necropsy to the Animal Health Laboratory (AHL) in Kemptville. The lambs were from five unrelated flocks in eastern Ontario. Crossbred lambs, as well as purebred Dorset, Suffolk, and Katahdin lambs were involved, and ranged in age from 5-16 weeks.

The onset of illness was reported as acute, and the most frequently reported clinical signs were excessive salivation described as "froth coming out of the mouth," depression and anorexia. Some lambs also had a nasal or ocular discharge. Lambs were non-responsive to various treatments, including topical and systemic antibiotics, and died 1-7 days after first observing clinical signs. On every affected premises, there had been more than one lamb with similar clinical signs. In these flocks, lamb mortality from all causes was reported to be between 1-15%.

At necropsy, there was severe multifocal to confluent erosion of the mucosa of the oral cavity, tongue, and esophagus. Multiple focal and larger areas of necrosis were found on the mucosa of the rumen, reticulum and omasum. In many cases, the necrotic oral, esophageal and forestomach mucosa was covered with a thick tan-yellow pseudomembrane. Sometimes, multifocal erosions were observed in the intestines.

Histology confirmed that all lambs had erosive mucosal disease. In some tissues, erosions were associated with perivasculitis, vasculitis and/or vascular thrombosis, but this was not a consistent finding. An unexpected histological finding in every lamb was acute to subacute, often severe, skeletal and cardiac myonecrosis. As there was no known exposure to ionophore coccidiostats, and liver selenium levels for all but one lamb were within the "adequate" range, the cause of the muscle damage was not explained.

One or more lambs from each of the five flocks was tested for Ovine herpesvirus 2 (OvHV-2, Sheep-associated malignant catarrhal fever of cattle virus) using the polymerase chain reaction (PCR) test, and all lambs were positive. Lambs tested negative for Border Disease virus using isolation, immunohistochemistry and serology, and bacterial culture results, including *Pasteurella trehalosi*, were negative. Tissue submitted to the CFIA was tested for foreign animal diseases with negative results.

Infection of the North American sheep population with OvHV-2 is common, so a positive PCR result does not prove that the agent caused the tissue damage. However, there is a report of experimentally infected sheep developing erosive to ulcerative mucosal lesions in the oral cavity and esophagus. In traditionally managed sheep flocks, lambs are infected with OvHV-2 by about 10 wk of age. They shed the virus in their nasal secretions between 6-9 months of age, several months after initial infection. These sheep maintain a reservoir of virus to infect other more susceptible ruminants, including cattle, bison and deer. The natural circumstances under which sheep show disease from this infection are not characterized, and it has been proposed that it may only occur in sheep with genetic or acquired immunological deficiency (Li, *et al.* 2005). In our cases, no specific or consistent immune deficiency was obvious, and the very young age of affected lambs was interesting.

At the time of publishing this article, the disease has re-emerged in one flock which had two affected lambs in 2008. The AHL and OMAFRA are keen to investigate more cases of sheep with these signs and lesions.

Li H, O'Toole D, Kim O, Oaks JL, Crawford TB. Malignant catarrhal fever-like disease in sheep after intranasal inoculation with ovine herpesvirus-2. J Vet Diagn Invest 2005 Mar; 17(2):171-175.

Selected Zoonotic Pathogens and Diseases Identified at the AHL, 2008

Beverly McEwen, Durda Slavic, Davor Ojkic, Josepha DeLay, Hugh Cal, and Margaret Stalker, Animal Health Laboratory (AHL), University of Guelph

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Many new, emerging, and re-emerging diseases of people are caused by pathogens originating from animals, or are shared between people and animals. The AHL plays an important role in public health by identifying zoonotic pathogens (**Tables 1** and **2**). These are numerator data reliant upon submission biases to the diagnostic laboratory and cannot be regarded as population prevalence estimates. Monitoring programs are not included.

***Mycobacterium tuberculosis*, was identified in a dog submitted for necropsy and details of this case are reported in the December 2008 AHL Newsletter.** The zoonotic pathogens most frequently identified at the AHL since 1999 are *Leptospira* spp., *Salmonella* sp., *Streptococcus* sp., and *Cryptosporidium* sp. Occupational exposure to pigs and horses is a risk factor for *S. suis* and *S. zooepidemicus* infections. Sporadic cases of dermatophytosis, cryptococcosis and blastomycosis are identified microbiologically and/or on cytology or histological sections.

In previous years, the numbers of isolates were tabulated; however, due to the increasing number of tests for selected pathogens, the number of cases will now be documented. For data prior to 2008, please refer to previous editions of the AHL newsletter.

Table 1. Cases with Selected Zoonotic Pathogens Isolated and/or Identified at the AHL, 2008

Agent	Bovine	Swine	Equine	Ovine	Caprine	Chicken	Turkey	Canine	Feline	Other	2008
Bordetella bronchiseptica		27	1					10	5	9	52
Campylobacter coli/ jejuni/ fetus subsp. fetus	2			5				7			14
Chlamydophila sp.				3	5					2	10
Clostridium difficile	1	7	14						1	2	25
Coxiella burnetii (Q fever)				2	13						15
Cryptosporidium sp.	127	4		5					1	7	144
Eastern equine encephalitis virus			10							2	12
Giardia sp.	10	3			1			38	3	1	56
Listeria monocytogenes	4	3		3	3					1	14
Methicillin-resistant *Staphylococcus aureus*			49					1		1	51
Mycobacterium tuberculosis								1			1
Rabies			1							3	4
Salmonella sp.	83	58	75	2	1	36	11	4	4	48	322
Streptobacillus moniliformis (Rat Bite Fever)										1	1
Streptococcus suis	4	153			1						158
Streptococcus equisimilis		41	26					8		1	76
Streptococcus zooepidemicus	1	5	96					5		1	108
Toxoplasma sp.				4	2				2		8
West Nile virus			2							68	70
Yersinia enterocolitica		6									

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Table 2. *Leptospira* spp. Seropositive Cases Identified at AHL, 2008, Microscopic Agglutination Test (MAT)

Leptospira spp. serovar	Bovine	Swine	Equine	Canine	Other & Not Specified
L. autumnalis	7	4	9	31	4
L. bratislava		5	7	8	
L .canicola	9	4		5	
L. grippotyphosa	12	5	2	47	
L. hardjo	5	3			
L. icterohaemorrhagiae	12	5	4	11	1
L. pomona	29	5	15	38	
Total	74	31	37	140	5

Domestic Surveillance and International Trade

Bruce McNab, Veterinary Science and Policy Unit, OMAFRA

Ontario's ability to provide quantitative data demonstrating effective animal health surveillance, including normal and abnormal patterns and trends, is becoming increasingly important to support trade and program funding. In addition, Animal Health Laboratory personnel need accurate case-history and demographic information to properly select and interpret laboratory tests and gross pathologic findings. The absence of this information affects the quality of the diagnostic service that is provided and, by extension, the ability to monitor disease trends in the province.

History, animal location, demographic information, and test results, are important for effective surveillance of animal health. Valid surveillance programs are increasingly necessary to support free trade, which is crucial to the Canadian livestock industry. Completely filling out the demographic and case-history information on AHL submission forms will provide one component of an effective surveillance system that can improve animal health in Ontario and support our exports internationally.

Attention: Ontario Large Animal Veterinarians

Janet Alsop, Veterinary Science and Policy Unit, OMAFRA

Please advise your clients of funding (up to $20,000 per producer, with 75 percent of an applicant's eligible costs reimbursed) now available under the Food Safety and Traceability Initiative (FSTI), which is part of Growing Forward, a federal-provincial-territorial initiative. The FSTI will provide funding to support the implementation of recognized food safety programs, e.g., CQA®, through the purchase and installation of equipment and/or the training of employees. **Producers should confirm eligibility of the project before making purchases**. Applications can be completed online (see below).

Equipment purchases that **may be** eligible for funding include:

- Scales and handling chutes (to determine that animal weights are accurate for antibiotic dosing)
- Pasteurizers (to destroy pathogens in swine liquid feeding systems)
- Feed milling systems (to improve accuracy of feed medication additions)
- Refrigerators
- Medicators
- Pressure washers

Any product or training that could improve food safety may be eligible. The program is "First come, First served," so producers should submit applications early.

For more information on the Food Safety and Traceability Initiative , please visit *www.omafra.gov.on.ca/english/infores/foodsafe/fsinitiative.htm*

For more information on Growing Forward, refer to *www.omafra.gov.on.ca/english/about/growingforward/whatsnew.htm*

Comparison of Saliva and Sera for RT PCR Testing for PRRS Virus

Tim Blackwell, Veterinary Science and Policy Unit, OMAFRA; George Charbonneau, Swine Services Group; and Al Scorgie, Tavistock Veterinary Services

It is sometimes necessary to monitor a farm or production area on a farm for the presence of Porcine Reproductive and Respiratory Syndrome virus (PRRSV). This is most commonly done through blood sampling and testing of the sera for PRRSV. However, blood collection from swine is not a simple task and usually requires two people to accomplish it. Recently, reports have indicated that saliva samples from infected pigs can produce results similar to sera (**Ceptor**, March, 2009). Saliva samples are most commonly collected by hanging cotton ropes in pens of pigs for 30 to 90 minutes and allowing the pigs to chew on the ropes. Ropes are then collected into plastic freezer bags and the saliva that has soaked into the ropes is wrung out into the bags. The saliva is transferred to serum containers and sent for reverse transcriptase polymerase chain reaction (RT PCR).

Recently, a small project was completed on nine Ontario swine farms to compare saliva testing to serum testing to identify PRRSV by RT PCR. Project farms were selected based on a suspicion that PRRSV was circulating in at least one area on the farm. All farms were sampled once with the exception of farm 9 that was sampled on two occasions approximately two months apart. Five blood samples were collected from each pen of pigs and approximately one meter of 2.5 cm cotton rope was hung in each pen where blood samples were collected. The five sera samples collected from each pen were combined into 2 pools (3 samples in one pool and 2 in the other) except in farm 9b, where all five sera samples were combined into one pool. Results are presented in **Table 1**.

One unexpected result from this trial was the higher than anticipated number of farms where circulating PRRSV was not identified in either sera or saliva samples although its presence was strongly suspected by either the owner or the veterinarian. On farms where neither sera nor saliva samples were positive by RT PCR, IDEXX ELISA antibody assays were performed, which verified a lack of sero-conversion in these pigs to PRRSV. PRRSV was identified on three (3) of the nine (9) farms in the study. Agreement at the pen level of comparison was quite variable although agreement at the farm level of comparison was very good.

The use of saliva testing to identify PRRSV may be a useful addition to blood sampling when a producer or veterinarians wants to know the status of circulating PRRSV on a farm or in a specific production area on a farm. The saliva collection technique is simple to perform and is cost effective. As seen in Table 1, agreement between sera and saliva results can vary. However, if the goal is to identify the presence or absence of virus, rather than to accurately estimate prevalence, the use of cotton ropes may be useful. A positive result on saliva testing is most likely accurate. On farms where the prevalence of PRRSV is suspected to be low, blood sampling is recommended to confirm negative results from saliva testing.

Table 1. Comparison of RT PCR Results Between Sera and Oral Fluid Samples Collected from Pigs in the Same Pens on Nine Different Farms

	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	Farm 6	Farm 7	Farm 8	Farm 9a*	Farm 9b
Sero +ve or susp.	0/4 pools	0/4 pools	6/10 pools	4/6 pools	0/8 pools	0/4 pools	0/13 sera	0/40 pools	10/26 pools	8/18 pools
Sero -ve	4/4 pools	4/4 pools	4/10 pools	2/6 pools	8/8 pools	4/4 pools	13/13 sera	40/40 pools	16/26 pools	10/18 pools
Oral Fluids +ve or susp.	0/2 fluids	0/2 fluids	2/5 fluids	2/3 fluids	0/4 fluids	0/2 fluids	0/3 fluids	0/20 fluids	4/13 fluids	3/18 fluids
Oral Fluids -ve	2/2 fluids	2/2 fluids	3/5 fluids	1/3 fluids	4/4 fluids	2/2 fluids	3/3 fluids	20/20 fluids	9/13 fluids	15/18 fluids
Pen Agreement	100%	100%	60%	100%	100%	100%	100%	100%	85%	38%
Farm Agreement	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%

* Farm 9 was tested on two occasions. Results for 9a were collected in December, 2008, and results for 9b were collected in February, 2009.

A Trial to Assess the Efficacy of *Streptococcus suis* Treatment Protocols

Janet Alsop, Veterinary Science and Policy Unit, OMAFRA, and Zvonimir Poljak, Ontario Veterinary College, University of Guelph

Morbidity related to *Streptococcus suis* is common in nursery age pigs on Ontario swine farms. Some farms or production systems experience a higher level of mortality than others. With the assistance of two swine practitioners, four nursery barns, ranging in size from 1,500 to 2,500 head and reporting significant problems with *S. suis*-related disease, were identified, and the producers involved agreed to cooperate in a trial. *S. suis* infection was confirmed by sample submissions from all of the barns.

The goal of the project was to determine whether the type of treatment administered influenced recovery and mortality rate. Three treatment protocols – procaine penicillin G (1 cc per 10 kg for three days) alone, procaine penicillin G plus isoflupredone (0.2 cc per 10 kg for two days) and procaine penicillin G plus isoflupredone plus nursing care – were used and the barn staff was asked to randomly assign affected pigs to a treatment. Staff recorded the age and weight of animals affected, clinical signs, treatment used, duration of treatment and outcome on a data recording sheet.

Participants were provided with an injectable steroid (isoflupredone) and materials for nursing care - a heat lamp, 60 cc syringe for administering water and a rubber mat (one quarter of a cow stall mat). Injectable procaine penicillin G was not provided since all producers already had this on hand. Removing affected pigs from pens, providing them with a warm, draft-free location and assisting them to drink is inexpensive and does not require much time or labour. The total cost per farm of the materials for nursing care was $51.15, or 2.5 cents per pig in a 2,000 head nursery. All of these materials can be re-used many times, which lowers the cost even further.

There were 106 treatments and outcomes recorded. In order to provide more statistical power, data were simplified by combining outcome into completely recovered (full value feeder pig) or not completely recovered, treatment into steroid or no steroid, and initial disease severity into two categories – severe (including convulsions) or less severe. Although there was no overall effect of treatment on the outcome, when the cases were stratified by disease severity, there was a tendency for a lower risk of mortality or culling in severe cases that were treated with a steroid. Because of the small sample size, this value was not statistically significant. Selection bias was also an issue. Barn staff preferentially selected the most severe cases to receive steroid treatment – 81% of severe cases were treated with the steroid whereas only 52.9% of the less severe cases were treated with the steroid. In field trials, it can be difficult to achieve random selection because of bias.

However, the trial demonstrated that it is worthwhile investing time and money on *S. suis*-affected pigs. Sixty three (59.4%) of the treated pigs recovered fully and were transferred to the finishing stage. With a current market value of approximately $34.00 per feeder pig, an investment per treated pig of approximately twenty-six cents for pharmaceuticals (in an 8-kg pig) and less than three cents for materials appears to be very cost-effective.

In trials with a larger sample size and with truly random treatment, it may be possible to demonstrate statistical significance in treatment outcomes. Practitioners who have clients who may be interested in participating in future trials are encouraged to contact Dr. Alsop (janet.alsop@ontario.ca or (519) 846-3420).

Effective treatment protocols will result in improved animal welfare, a reduced risk of antimicrobial resistance, decreased economic losses and reduced risk of zoonotic transmission.

Glycantyping – Implications for PRRSV Classification and Vaccinology

Janet Alsop, Veterinary Science and Policy Unit, OMAFRA, and Sue Burlatschenko, Goshen Ridge Veterinary Services

Porcine Reproductive and Respiratory Syndrome virus (PRRSV) strain differentiation can be important for swine veterinarians. Methods, such as open reading frame 5 (ORF5) sequencing and restriction fragment length polymorphism (RFLP) analysis, indicate strain variability but not how the strains differ immunologically [1]. Researchers in Iowa and North Carolina have recently proposed a new classification scheme for PRRSV strains [2].

The main structural proteins of PRRSV are the nucleocapsid (N) protein, the membrane/matrix (M) protein and the main envelope glycoprotein 5 (GP5) [3]. GP5 is involved in virus assembly, and also appears to be involved in the entry of virus into susceptible host cells [4].

Pigs mount a rapid antibody response to infection by PRRSV, but the antibodies are directed mainly against the N and M proteins and are non-virus neutralizing [3]. A number of studies have indicated that anti-GP5 antibodies play a major role in PRRSV neutralization in both North American and European PRRSV strains [4]. Glycans are chains of sugar molecules that extend outward from GP5, on the virus membrane. These glycans have the ability to change the way in which the virus is recognized by the host, by masking key epitopes, as well as creating new epitopes, on the virus membrane [1]. This is called 'glycan shielding', which is a defence mechanism used by the virus to mask it from the host's immune system and it may explain the delayed and weak immune response observed in PRRSV-infected animals [4].

Vander Veen, *et al.* have shown that glycantype is a better predictor of virus neutralization than sequencing or RFLP pattern [2]. PRRSV strains with fewer glycans on GP5 induce a more rapid neutralizing antibody response compared with strains that are highly glycosylated [1,2,4]. PRRSV strains with three or more glycans are less amenable to neutralization.

Erdman, *et al.* have classified PRRSV strains according to the number of glycan linkages [1]. Strains with two N-linked glycans on GP5 (designated NA2) are lower in virulence, but are rare. Field strains of PRRSV in North America switch between NA3 and NA5 [5]. The researchers have also found that, when two NA2 strains were used to infect gilts, high virus neutralizing titres were obtained [2]. However, at this time, there are no commercial vaccines that fall into the NA2 group [5]. The researchers have data indicating that there is heterologous protection against PRRSV strains resulting from vaccination with a vectored PRRSV GP5 vaccine [5].

1. *Erdman MM, Laniewski L, Mogler M, Vander Veen RL, Harris DLH. Glycantype: A new classification scheme for PRRSV. Proc Iowa State Univ Swine Disease Conf Swine Pract. Ames, Iowa. 2008:81-84.*
2. *Vander Veen RL, Erdman MM, Mogler M, Laniewski L, McVicker J, Owens G, Kamrud K, Harris DLH. Preparation of GP5-M heterodimer glycantype specific recombinant protein and replicon particles. Proc AASV. Dallas, Texas. 2009:99-101.*
3. *Faaberg KS, Hocker JD, Erdman MM, Harris DLH, Nelson EA, Torremorell M, Plagemann PGW. Neutralizing antibody responses of pigs infected with natural GP5 N-glycan mutants of porcine reproductive and respiratory syndrome virus. Viral Immunol 2006; 19:294-304.*
4. *Ansari IH, Kwon B, Osorio FA, Pattnaik AK. Influence of N-linked glycosylation of porcine reproductive and respiratory syndrome virus GP5 on virus infectivity, antigenicity, and ability to induce neutralizing antibodies. J Virol 2006; 80:3994-4004.*
5. *Harris DLH. Personal Communication.*

Now Available—CVMA Antimicrobial Prudent Use Guidelines for Beef Cattle, Dairy Cattle, Poultry and Swine

Janet Alsop, Veterinary Science and Policy Unit, OMAFRA

These guidelines provide evidence-based antimicrobial treatment protocols for many of the common bacterial infections treated in beef and dairy cattle, poultry and swine, and are a useful reference for veterinary practitioners and students. CVMA members can access the electronic version on the CVMA website, *http://canadianveterinarians.net*. All large-animal and mixed-animal practices in Canada will receive a hard copy of the guidelines by mail and, if they are CVMA members, they can request additional copies at no charge.

Antibiotics as Loss Leaders?

Kathy Zurbrigg, Veterinary Science and Policy Unit, OMAFRA

A summary of "US supermarkets redefine antibiotic misuse," Lancet Infectious Diseases 2009; 9 (May):265

Loss Lead is a marketing concept where an item is offered for sale, at or below cost, to *lead* to the subsequent sale of other items to customers drawn to the store to purchase the loss lead. The concept has been practiced by grocery stores for decades. Recently the practice has been extended to antibiotics by some stores in the US.

In late 2008, US grocery store chains Giant, Publix, Stop and Shop and Wegman's announced a program offering generic versions of various antibiotics free of charge for patients with a prescription. The program began under the guise of "goodwill" during the economic downturn and was scheduled to end in March 2009; however, several of the chains extended their promotions. This practice is likely to lead to a misuse of antibiotics and is a giant step backwards in the fight against antibiotic resistance. With this promotion, customers are likely to put more pressure on physicians to prescribe antibiotics. Even if there is only a small chance of antibiotics resulting in recovery from a health problem, physicians may be more likely to write a prescription if the patient does not have to pay for the drugs. The offer of free antibiotics may also promote the stockpiling of unused medications, the sharing of medications with family members or friends with "similar" self-diagnosed problems, and not following the labeled directions for the course of the antibiotics.

European studies have already shown an association between increased use, over the counter availability of antibiotics, and the increased resistance of human pathogens to widely used, cheap antibiotics. These findings prompted the EU to hold an antibiotic awareness day, to educate patients and re-educate physicians about the use and misuse of antibiotics. If antibiotics are allowed to be used as loss leaders, then retail chains should be responsible for promoting a similar education campaign.

Assessment of Subclinical Venous Catheter-related Diseases in Horses and Associated Risk Factors

Paisley Canning, Summer Student, Veterinary Science and Policy Unit, OMAFRA

The insertion of an indwelling venous catheter provides a stable and secure access point to a vein. However, the use of indwelling catheters also carries a risk of morbidity for the horse. Complications, such as thrombus formation, septicemia and thrombophlebitis, can develop at the catheter site and increase recovery time. Clinical signs such as fever, thickening of the venous wall, and evidence of pain on palpation are signs of thrombophlebitis. Early detection of thrombus formation and removal of associated catheters will reduce the risk of thrombophlebitis and associated problems for the horse. Specific tests may be useful for early detection of thrombus formation as clinical signs have poor sensitivity.

Geraghty, *et al.* (2009), in their recent study in the *Veterinary Record*, assess the use of microbiological and ultrasonographic examinations to identify early evidence of thrombophlebitis. The authors also identified risk factors that may impact the development of disease at catheter sites. In their study, 119 intravenous catheters were introduced using aseptic techniques into the left or right jugular vein of 102 horses for the administration of treatment. Thrombus formation was considered a subclinical sign of thrombophlebitis. Ultrasonographic examination was used to measure jugular vein thickness every 48 hours. This was carried out on 98 catheterized veins in 89 horses (some horses were unable to safely walk to the ultrasonography room). Thrombosis was detected in 16 veins (16.3%) in 15 of the 89 horses. Thrombus formation appeared 24 hours after catheterization as a hyperechogenic irregularly shaped mass attached to the venous wall and did not resolve while the catheter was in place. Additionally, samples from 92 catheter sites were collected and cultured for microbes. Twelve (12) catheter sites were positive on culture, but there was no association between the presence of bacteria at the catheter insertion site and venous thickening.

(Continued on page 11)

Two risk factors, rectal temperature and administration of NSAIDs, were significantly associated with the detection of thrombosis. If a horse's rectal temperature was greater than 38.5°C at catheterization, it was four times more likely to develop thrombosis (as identified ultrasonographically). Fever can indicate a systemic inflammatory response associated with infection and thrombus formation.

Conversely, there was a negative association between the administration of NSAIDs via catheter and thrombus formation. NSAIDs may reduce some of the vascular effects of severe systemic disease and consequently impede thrombus formation. Other risk factors, such as age, breed, heart rate, season of admission and bacterial colonization of the catheter, were not predictive of subclinical thrombophlebitis.

It is impractical for veterinarians in the field to monitor all catheter insertion sites ultrasonographically for subclinical signs. However, for horses at a high risk for thrombophlebitis (fever, immunocompromised, severe infection) ultrasonography may assist in the early detection of thrombophlebitis and reduce the risk of increased or prolonged morbidity. It may be beneficial to relocate catheters after 24 hours in high-risk horses as this study demonstrates thrombus formation is occurring at this time.

Dolente BA, Beech J, Lindborg S, et al. Evaluation of risk factors for development of catheter-associated jugular thrombophlebitis in horses: 50 cases (1993-1998). J Am Vet Med Assoc 2005; 227 (7):1134-1141.

Geraghty TE, Lovem DJ, Tayler J, et al. Assessment of subclinical venous catheter-related diseases in horses and associated risk factors. Veterinary Record 2009; 164:227-231.

Tan R, Dart AJ, Dowling BA. Catheters: a review of the selection, utilisation and complications of catheters for peripheral venous access. Aust Vet J 2003; 81 (3):136-139.

Testing Forages for Mycotoxins and their Effect on Horses

Bob Wright, Veterinary Science and Policy Unit, OMAFRA

Occasionally practitioners are concerned that vague clinical signs are related to mycotoxins in horse feed. Mycotoxins are secondary metabolites produced by fungi and affect various animals upon ingestion or inhalation. Horses may ingest mycotoxins in contaminated grain (e.g., commercially prepared feeds containing wheat middlings and wheat bran) as well as in their pasture, hay and bedding. Pasture grasses, hay, grain, straw and stubble can all support the growth of various fungi. The fungi can exist as saprophytes, living on the outside of the plant and obtaining nutrients from the plant with no benefit to the plant, or exist as endophytes within the plant in a symbiotic relationship, providing benefits to the plant while obtaining nutrients from the plant. The saprophytes include the more common genera *Aspergillus, Claviceps, Stachybotrys, Fusarium* and *Penicillium*. The endophytes live between the plant's cell walls and include the more common genera *Balansia, Epichloe, Acremonium* and *Neotyphodium*.

Fungi and their associated mycotoxins are present on grain crops in varying amounts each year, depending on the climatic growing conditions. A cool, wet, growing season increases the likelihood that fungi, especially *Fusarium* and its mycotoxins, will be present in small grains. The high moisture level in grain encourages fungal growth while the cool temperatures increase the production of mycotoxins.

Sampling and Analysis

When veterinarians are concerned that vague health issues may be related to above normal mycotoxin concentrations, all feedstuffs should be tested, including the bedding. Feed and bedding commonly contain a background low concentration of fusarium mycotoxins. This should preferably be below 1 ppm. Samples of hay and straw are taken using a core sampler from at least 10 bales. Representative samples of all grain and hay/straw samples should half fill a 10-inch square ziplock bag. All bags

(Continued on page 12)

should be marked with the manufacturing date, lot and supplier. Hold samples in the freezer prior to testing. For cases potentially involving lawsuits, take triplicate samples. Send one to the laboratory for testing, leave one with the owner to keep in the freezer and keep the third in your own freezer for further testing, should it be required.

Laboratory testing for mycotoxins is preferably performed using HPLC (high-performance liquid chromatography) or GC (gas chromatography) methodology rather than using one of the quick ELISA tests. ELISA (enzyme-linked immunosorbent assay) assays are only validated for testing raw grains. The tolerance levels of mycotoxins suggested for feed can be used to determine the potential risk when using hay and straw bedding. Refer to the information sheet *Molds, Mycotoxins and their Effect on Horses* on the OMAFRA website at *www.omafra.gov.on.ca/english/livestock/horses/facts/info_mycotoxin.htm*

Consult with the laboratory prior to testing. Laboratories vary in their capacity to analyze for mycotoxins. Samples may need to be sent to external laboratories.

The Animal Health Laboratory (AHL) at the University of Guelph offers some toxicology using GC and HPLC and is currently increasing its capacity. Check with Dr. Brent Hoff at (519) 824-4120 ext. 54527 for details.

North Dakota Veterinary Diagnostic Laboratory offers extensive mycotoxin testing using GC/MS at affordable rates. A screening for vomitoxin, zearalenone, 15 trichothecenes along with aflatoxin and fumonisin is priced at $90 (US funds) or without aflatoxin and fumonisin for $55. Details can be found at *www.vdl.ndsu.edu/vdl/FeeSchedule.aspx?rf=services&sf=giv*

Laboratory tests for ergovaline (the ergot alkaloid of fescue toxicity) and lolitrem B (the ergot alkaloid of perennial ryegrass) are available from the Endophyte Service Laboratory at Oregon State University. Refer to *http://oregonstate.edu/endophyte-lab/* for details. Thousands of samples are analyzed at this laboratory annually for ergovaline using HPLC. Analysis for other ergopeptine alkaloids is limited due to the lack of control standards.

Samples can be sent to US labs directly or through arrangements with the AHL. Direct couriering to the USA requires customs clearance and some attention to detail as indicated below.

USDA and Customs Requirements for Feed Samples

Sending hay samples through the US border is usually not a problem. However, grain and commercially prepared feeds require added documentation. Attach an envelope to the shipment which includes the following information in triplicate:

1. A statement on the courier form that value of the shipment is less than $2.00
2. A list of all samples
3. An import permit from the US laboratory. Call the laboratory and ask for a copy of their import permit. These are issued annually covering all feed samples going to the lab.
4. For commercially prepared feeds, include an ingredient list supplied by the manufacturer.
5. A covering letter indicating that the samples;
   a. Are intended for laboratory analysis only.
   b. Do not contain seed intended for planting.
   c. Do not contain any animal bi-products.
   d. Will be destroyed once analysis is completed.

Once the results are received from the laboratory, the challenge will be to interpret them. Research involving the effect of mycotoxins on horse health is limited when compared to research in other species, such as cattle and swine. The information sheet - *Molds, Mycotoxins and their Effect on Horses* will help provide some guidelines.

What Agriculture Needs to Understand: Do Not Belittle your Customers.

Kathy Zurbrigg, Veterinary Science and Policy Unit, OMAFRA

Adapted from "What Agriculture must understand" (Feedstuffs, April 20, 2009) by Dr. Bernard E. Rollin, Professor, Colorado State University, Ft. Collins

Change is coming to the management of livestock industries and it is being driven by consumers. Regardless of the industry, a common target is the need for change in livestock housing, which would provide the animals greater freedoms; for example, replacing the use of sow stalls, veal crates and the use of battery cages for hens with loose housing systems.

The agricultural industry occasionally portrays societal concerns about the treatment of animals in agriculture, as driven by vegetarian activist extremists who are determined to destroy livestock industries worldwide.

The portrayal of the problem as one of animal activism does not fit with current observations. Proposition 2 in California (a state referendum on passing legislation that bans the use of sow stalls, veal crates and battery cages) passed with 67% of the vote. No one believes that the majority of people who voted for it are vegans or vegetarians. They are people who consume animal products but are concerned about how those products are produced. They are no more out to destroy animal agriculture than the people who worry about steroid use in baseball are out to destroy baseball. Rather, they are sufficiently concerned about how animals are raised that they voiced their concern even in the face of threats that food prices would go up. People will not even give up meat, milk and eggs when told to do so by their physicians based on claims that their health is at risk, so they certainly won't do so because some vegans tell them to.

Certainly, activists do attempt to sway public opinion in favour of their agenda, but they do so by appealing to concerns already there in the general public. And surely, while they hope that more people will become vegan, the chance of moving large numbers of people to radically change their eating habits is vanishingly small.

The Environmental Impact of Dairy Production: 1944 Compared with 2007

J. L. Capper*, R. A. Cady† and D. E. Bauman*,2

An abstract from Journal of Animal Science, 2009:V89: pg 2160-2167

**** Department of Animal Science, Cornell University, Ithaca, NY 14853; and † Elanco Animal Health, Greenfield, IN 46140***

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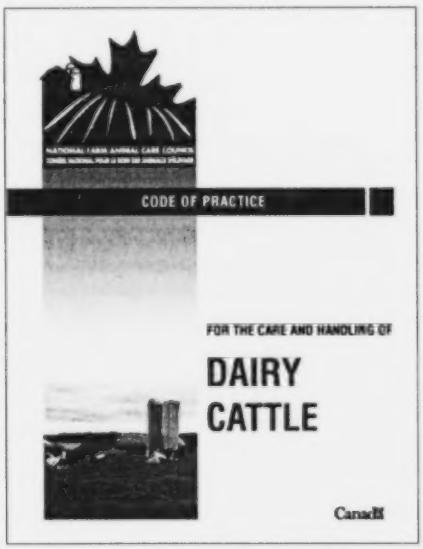
A common perception is that pasture-based, low-input dairy systems characteristic of the 1940s were more conducive to environmental stewardship than modern milk production systems. The objective of this study was to compare the environmental impact of modern (2007) US dairy production with historical production practices as exemplified by the US dairy system in 1944. A deterministic model based on the metabolism and nutrient requirements of the dairy herd was used to estimate resource inputs and waste outputs per billion kg of milk. Both the modern and historical production systems were modeled using characteristic management practices, herd population dynamics, and production data from US dairy farms. Modern dairy practices require considerably fewer resources than dairying in 1944 with 21% of animals, 23% of feedstuffs, 35% of the water, and only 10% of the land required to produce the same 1 billion kg of milk. Waste outputs were similarly reduced, with modern dairy systems producing 24% of the manure, 43% of CH_4, and 56% of N_2O per billion kg of milk compared with equivalent milk from historical dairying. The carbon footprint per billion kilograms of milk produced in 2007 was 37% of equivalent milk production in 1944. To fulfill the increasing requirements of the US population for dairy products, it is essential to adopt management practices and technologies that improve productive efficiency, allowing milk production to be increased while reducing resource use and mitigating environmental impact.

A Twenty Percent Solution for Dairy Calves

Neil Anderson, Veterinary Science and Policy Unit, OMAFRA

The Code of Practice for the Care and Handling of Dairy Cattle (2009) includes ***requirements*** and ***recommended best practices***. For unweaned calves, a requirement states that *calves must receive a volume and quality of milk or milk replacer to maintain health, growth and vigor.* To achieve the requirement, a best practice recommends that producers *offer calves a minimum total daily intake of 20% of body weight in whole milk (or equivalent nutrient delivery via milk replacer) until 28 days of age (e.g., approximately eight litres per day for Holstein calves).*



Since ten percent of body weight (BW) has been the milk-feeding guideline for more than half a century, doubling the volume may worry some calf feeders. Nevertheless, the twenty-percent guideline is a *best practice* that meets the needs of the calf for its health and welfare. At meetings this winter, a few recent adopters gave testimonials about improved health and growth of their calves fed 8 or 10 litres. It was new news to most but old news to a few in the audience who have been twenty-percent-feeders for several years.

When feeding by the Code, a 40 kg calf should be offered eight litres of milk per day and bob calves averaging 50 kg should be offered about 10 litres.

Data collected at a few producer meetings have shown that 70% of participants feed five litres or less in the first week and 55% feed six litres or less in the second week of their calves' lives. To put the recommendations into perspective, the majority of producers surveyed were feeding 25-50 percent less than the recommended best practice.

Several Ontario practitioners are championing the feeding of more milk to newborn calves. However, an intensive campaign may be needed to facilitate or speed adoption of the 20% of BW practice by a majority of producers. With the release of the new Code, this is the year to work with your producers, local milk committees, management clubs or feed company representatives to accelerate implementation. A target of 75% compliance with the feeding guideline by June 2010 is reasonable. The 20% of BW practice may be the solution for healthier and more productive calves for your clients.

Soon, Dairy Farmers of Canada, (613) 236-9997, will print and distribute the new Dairy Cattle Code to producers. The document is available from the National Farm Animal Care Council (NFACC) on their website— *www.nfacc.ca* . It is also available in French.

Klebsiella Mastitis in Dairy Cattle— Updating the Dogma

Ann Godkin, Veterinary Science and Policy Unit, OMAFRA

Recent research and comments from other advisors suggests that interest in Klebsiella mastitis is high. As we head into the warm seasons of the year, it is a good time to take stock of what we know and do not know about this pathogen.

Klebsiella is a gram-negative bacterial organism. Klebsiella mastitis occurs sporadically following transmission from the cow environment to the teat end. However, the occurrence of outbreaks and the persistence of Klebsiella as a major, repeated cause of mastitis on some farms infers that there are cow and farm factors that influence the success of these bacteria as a mastitis infection.

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Clinical mastitis caused by *Klebsiella* sp. has been shown to be more common in herds with low bulk milk SCCs (less than 150,000 cells/ml) in a survey from the Netherlands [1]. At the Quality Milk laboratories in New York, information from about 400 annual herd survey cultures done between 1992 and 2004, has shown that Klebsiella was isolated with an increasing frequency from cow milk samples. After correction for increasing herd size, the proportion of herds with at least one Klebsiella isolate rose from about 5% of herds to close to 25% of herds. Over this 12-year period, herd infection rates also rose from an average of about one cow per herd to about three. The variability in the number of infected cows per herd was large and also increased over time. Some herds had very high infection rates, with close to 25% of samples positive for Klebsiella [2].

Cow manure is the source of Klebsiella on most farms. In a study of 100 healthy cows followed for five months, Klebsiella was found in 80% of fecal samples. In 10 herds surveyed at a single time, over 80% of manure samples were positive for Klebsiella. This suggests that Klebsiella is present in cow manure on many farms [3]. Fecal shedding of Klebsiella by cows is very common, but cows have been shown to be transiently rather than persistently colonized by these bacteria [4].

Overall, the genetic diversity of the strains of Klebsiella isolated from mastitis is large [5] and little is known about differences in strains with regards to mastitis severity, bacterial virulence, etc. Other work has shown that strains of Klebsiella are diverse between herds, but that strains are somewhat consistent within herds.

A study culturing soil samples from one New York farm found Klebsiella in almost all samples from corn and alfalfa plots that received manure. Klebsiella was also detected in corn at the time of harvest and in TMR samples taken from the feed alley [6]. These researchers suggest that Klebsiella strains "cycle" through the digestive tracts of cows on a farm after they are fed crops fertilized with cow manure, and are excreted in manure, leading to contamination of the cow bedding and environment. They hypothesize that increased starch levels in dairy cow diets and higher rates of passage through the bovine GI tract may increase fecal shedding of Klebsiella by cattle [6]. Further work is needed to explore this.

Bedding with shavings is frequently associated with Klebsiella mastitis. While isolations of Klebsiella from unused shavings are extremely rare, the numbers of Klebsiella rise rapidly after bedding is in use. Klebsiella counts rose even more quickly than *E. coli* in one study of dairy cow bedding. Moisture and warmth are essential for rapid Klebsiella multiplication. Klebsiella counts in sawdust and shavings rose over several days in proportion to a decrease in the dry matter content of the bedding [7]. Klebsiella is notorious for being present in high numbers in used bedding that visibly appears clean but may have high moisture content.

The risk of Klebsiella mastitis is not linked only to the use of shavings or sawdust. Klebsiella bacteria can be found in other types of bedding as well [8]. Sand, although inorganic, can also have high Klebsiella numbers shortly after it is added and used [2]. Any bedding contaminated with manure and held in the right environmental conditions has the potential to have high Klebsiella numbers.

Antibiotic therapy is unsuccessful in removing Klebsiella from the udder. Chronic mastitis and high cow SCCs persist even if clinical illness is not severe. Prevention of infection, not reduction in clinical signs, is the major pathway to improvement in Klebsiella mastitis rates and impact. No specific vaccines are available for the prevention of Klebsiella mastitis.

Reducing Klebsiella contamination of the cow and the cow environment requires good manure management, including frequent manure removal (scraping) and bedding renewal (for all types of bedding). Excellent ventilation that provides good air movement will help to reduce humidity, which is particularly conducive to Klebsiella multiplication, in warm weather. Maintenance of a lower animal density in warm weather is a strategy that should be considered when annual herd problems occur.

In summary, overall rates of Klebsiella mastitis in herds and across jurisdictions should be monitored for a change in incidence and prevalence as is

(Continued on page 16)

believed to have occurred elsewhere. Klebsiella mastitis can become a significant issue in low bulk milk SCC herds. As the proportion of herds achieving low bulk milk SCCs and higher milk production rates increases we can anticipate more herds with Klebsiella mastitis problems if specific Klebsiella mastitis preventive measures are not implemented.

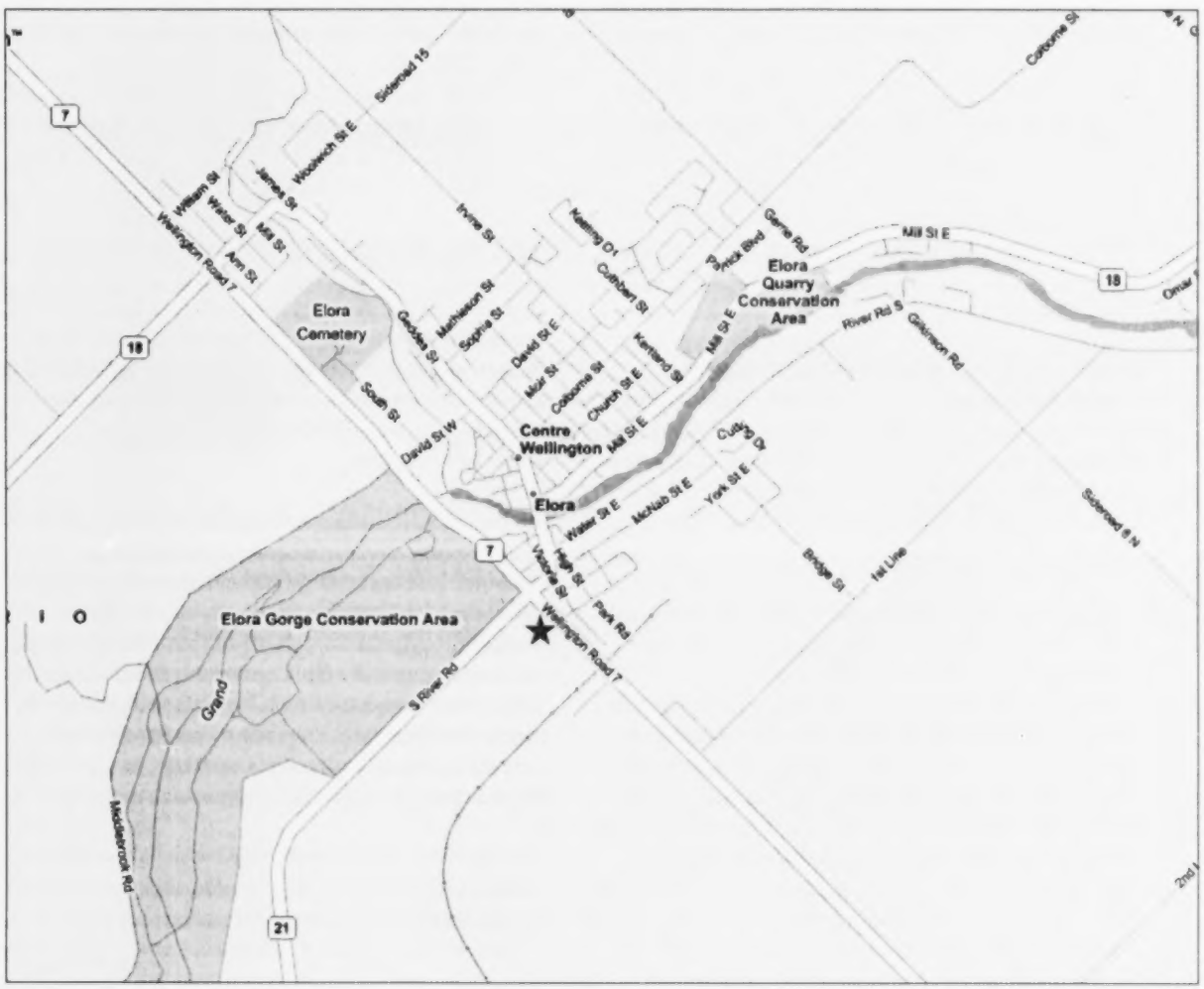
1. *Barkema, et al. J Dairy Sci 1998; 81:411.*
2. *Zadoks, et al. NMC Proceedings 2007.*
3. *Munoz, et al. J Dairy Sci 2006; 89:3425.*
4. *Munoz, et al. J Dairy Sci 2007; 90:1220.*
5. *Paulin-Curlee GG, et al. J Dairy Sci 2007; 91:554.*
6. *Zadoks, et al. NMC Proceedings 2008.*
7. *Zdanowicz, et al. J Dairy Sci 2004; 87:1694.*
8. *Godden, et al. J Dairy Sci 2008; 91:151.*

We were misinformed.

The unit number at our new location is Unit 10—not Unit 1—as indicated in the March issue of **Ceptor**. Our new address is:

6484 Wellington Road 7, Unit 10
Elora, Ontario N0B 1S0

Telephone numbers for staff remain the same. The fax number is (519) 846-8178.



Continuing Education/Coming Events

June 22 & 23, 2009	American Association of Swine Veterinarians Conference—Advanced Techniques for Swine Veterinarians "Optimizing Resources," Iowa State University, College of Veterinary Medicine, Ames, Iowa. *www.aasv.org/summerconf*
June 24 & 25, 2009	Ontario Pork Congress, Stratford Agricultural and Recreational Complex, Stratford, Ontario *www.porkcongress.on.ca*
June 25, 2009	Ontario Association of Swine Veterinarians Annual Meeting, Arden Park Hotel, Stratford, Ontario. *www.oasv.ca*
July 6-10, 2009	43rd Congress of the International Society for Applied Ethology, Cairns Convention Centre, Cairns, Australia. *www.isae2009.com*
July 7-9. 2009	International Conference on Bovine Mycoplasmosis, Saskatoon, Saskatchewan. *www.bovinemycoplasma.ca*
August 13, 2009	George A. Young Swine Health and Management Conference, Marina Inn, South Sioux City, Nebraska. *http://georgeyoungswineconference.unl.edu*
September 17-19 2009	2009 Joint Scientific Convention—Canadian Embryo Transfer Association (CETA/ACTE) and American Embryo Transfer Association (AETA), Hilton Montréal Bonaventure, Montréal, Québec *www.ceta.ca/convention. htm*
September 19-22 2009	Allen D. Leman Swine Conference, RiverCentre Conference Facility, Saint Paul, Minnesota. *www.cvm.umn.edu/outreach/events/adl/home.html*
September 20-24, 2009	International Dairy Federation World Dairy Summit, Maritim Hotel Berlin, Berlin, Germany. *www.wds2009.com*
September 30 - October 2, 2009	SafePork 8th International Symposium on the Epidemiology and Control of Foodborne Pathogens in Pork, Hôtel Château Laurier, Québec City, Québec. *www.safepork2009.org*
October 23 & 24, 2009	Ontario Association of Swine Veterinarians Fall Conference. *www.oasv.ca*
November 12 & 13 2009	Dairy Cattle Reproduction Council regional meeting, Crowne Plaza Riverfront, St. Paul, Minnesota. *www.dcrcouncil.org*
November 19 & 20 2009	Dairy Cattle Reproduction Council regional meeting, Doubletree Hotel Riverside, Boise, Idaho. *www.dcrcouncil.org*
March 6-9, 2010	American Association of Swine Veterinarians 41st Annual Meeting, Hilton Omaha Hotel, Omaha, Nebraska. *http://aasv.org/annmtg*
July 18-21, 2010	21st International Pig Veterinary Society Congress—Sharing Ideas-Advancing Pig Health, Vancouver Convention and Exhibition Centre, Vancouver, British Columbia. *www.ipvs2010.com*
November 14-18, 2010	26th World Buiatrics Congress, Espacio Riesco Convention Centre, Santiago, Chile. *www2.kenes.com/buiatrics2010/congress/Pages/General_Information.aspx*

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Unit 10, 6484 Wellington Road 7, Elora, ON N0B 1S0
Tel.: (519) 846-3418 Fax: (519) 846-8178 E-mail: kathy.zurbrigg@ontario.ca

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Deadline for next issue: August 14, 2009

Ontario
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